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14. ABSTRACT
The primary purpose of this grant is to determine the overall survival benefit in Stage IV HER2 positive breast cancer patients vaccinated with a HER2 ICD peptide-based vaccine while receiving maintenance trastuzumab. The scope of the work includes a Phase II single arm study of a HER2 ICD peptide based vaccine given concurrently with trastuzumab. Six patientshave been enrolled during the last reporting period. All adverse events reported for these six subjects are of low grade. Patients enrolled will be HER2 overexpressing stage IV breast cancer patients who have been treated to a clinical complete remission or have stable bone only disease and are within 6 months of starting maintenance trastuzumab. There have been no major findings to date.

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Phase II, Clinical Trial, HER2, Trastuzumab

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INTRODUCTION

The subject of this grant is to determine whether a HER-2/neu (HER2) intracellular domain (ICD) peptide vaccine, administered in combination with trastuzumab, will impact outcomes in patients with Stage IV HER2-positive breast cancer. The primary purpose of this grant is to determine the overall survival benefit in Stage IV HER2 positive breast cancer patients vaccinated with a HER2 ICD peptide-based vaccine while receiving maintenance trastuzumab.

The scope of the work includes a Phase II single arm study of a HER2 ICD peptide based vaccine given concurrently with trastuzumab. Patients enrolled will be HER2 overexpressing stage IV breast cancer patients who have been treated to a clinical complete remission or have stable bone only disease and are within 6 months of starting maintenance trastuzumab. The primary objective is an estimate of overall survival (OS) compared to a historical control of patients treated with chemotherapy and trastuzumab (55% at 2 years). We hypothesize that the overall survival rate at 2 years with vaccination, if successful, would be 75%. Fifty-two patients will provide 92% power to detect a statistically significant increased survival rate compared to the fixed historical rate of 55% at the one-sided significance level of p=0.05. Secondary objectives include the assessment of the toxicity of the combined approach as well as the immunogenicity of HER2 ICD peptide vaccination. If there is evidence to suggest that the true rate of Grade IV toxicity exceeds 5% or the true rate of Grade III-IV toxicity exceeds 10% then the trial will be stopped for safety concerns. Immunogenicity of the approach will be evaluated as the ability of the vaccine to elicit HER2 ICD specific T cell immunity, to elicit epitope spreading, and to stimulate both a CD4+ and CD8+ immune response. Immune response and epitope spreading will then be modeled as time-dependent covariates in Cox proportional hazards regression models for OS to assess the correlation of each of these outcomes with the hazard of mortality.

BODY

Task 1*: To assess the potential clinical impact of the administration of a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving concurrent trastuzumab monotherapy*

a. Reconstruct and vial the HER2 ICD peptide vaccine. This task has been completed. The vaccine product (lot 6002) continues to be monitored at specific intervals for product stability. A Stability Study Log for lot 6002 is maintained. The study log lists the testing dates and provides a summary table to record data for each time point tested. All reserved stability vials are stored under the same conditions as the final product, -20 ± 2°C. At each stability time point reserved vials are removed from storage and visually inspected for appearance. MALDI-TOF mass spectrometry and High Performance Liquid Chromatography (HPLC) are used to confirm the stability.

b. Enroll and treat patients. This study was officially approved by the US Army Medical Research and Materiel Command (USAMRMC) Human Subjects Research Review Board (HSRRB) on June 1, 2006. As reported in the last Annual Report (27 APR 2005 – 26 APR 2006) we had obtained all necessary approvals for this project through our institution and the FDA.

During the last reporting period we enrolled six subjects (started December 2006-April 2007). Table 1 demonstrates the study status of enrolled subjects through April 26, 2007.

Table 1. Study Enrollment Table

Study Time Point	Number of subjects completed to specified time point	Off Study
Vaccine 1	2	0
Vaccine 2	1	0
Vaccine 3	1	0

Study Time Point	Number of subjects completed to specified time point	Off Study
Vaccine 4	2	0
Vaccine 5	0	0
Vaccine 6	0	0
Total	**6**	**0**

To be eligible for this study, subjects must be enrolled within 6 months of initiating maintenance trastuzumab (Herceptin). As part of the last report submitted on May 26, 2006 we provided a list of 12 potential subjects with the date they began their maintenance Herceptin as well as the date on which they would need to be enrolled before the 6 months Herceptin treatment window had passed. Once this study was approved in June 2006 only one of the 12 subjects remained potentially eligible, and to participate she had to have enrolled by July 2006. Unfortunately, by the time all needed source documents (i.e., imaging, clinical labs, cardiac function tests) were collected, this individual had exceeded the 6 month maintenance Herceptin limit and was no longer eligible.

We have taken several steps to increase screening and enrollment for this study. These efforts include:

- A collaboration with the Seattle Cancer Care Alliance (SCCA) has been initiated to review patient records for potential trial eligibility at initial consultation visits. All physicians in the SCCA Breast Cancer Group are aware of the trial inclusion and exclusion criteria and on-site research coordinators provide study information to patients with a potential interest in study participation.

- We have an ongoing research collaboration with Breastlink, Inc., a large private oncology practice in Southern California, that exclusively treats breast cancer. Breastlink research coordinators regularly review their patient lists for Stage IV HER2 positive patients that are beginning trastuzumab therapy. Subjects interested in participating are provided with our Screening Coordinator's contact information. Since January 2005 about one third of the subjects enrolling in our group's studies have been referred by Breastlink. Two of the 6 patients currently on this study were referred by them and an additional 3 patients from the practice are currently being screened and are potentially eligible for study.

- Table 2 provides a list of Community Outreach activities performed by our group, The Tumor Vaccine Group during the last reporting period (27 APR 2006 – 26 APR 2007). Speaking engagements are usually performed by the PI, study physician or senior scientist from the research laboratory. The booths that we have at other speaking engagements can be manned by all members of the research team. Recuitment to this protocol is a priority for these presentations.

Table 2. List of Community Outreach Events

Name of Event	Description of Event	Date of Event
South Lake Union Poster Session (Seattle, WA)	Poster session in our building to showcase active work. Presented a poster on Recruitment Issues in Clinical Research	June 8, 2006
Site Meeting with Breastlink Inc. (Seattle, WA)	In person meeting with Breastlink Inc. staff to give an overview of the science and clinical trials we have active.	June 23, 2006
South Lake Union Block Party, Booth with UW Medicine (Seattle, WA)	Had a booth where we talked to the general public about immunotherapy and cancer	August 12, 2006
Ladies of Somerset Bridge Group (Bellevue, WA)	A group that gets together to raise money for breast cancer research by playing bridge. We attended as their	October 12, 2006

Name of Event	Description of Event	Date of Event
	guests and spoke about our group.	
Washington Sate Alliance for Healthy Communities of Color Conference, "Healthy Advocacy: Getting into the Game" (Kent, WA)	Networked with Minority Advocate Groups and set up a community talk about cancer clinical trials	November 17, 2006
Continuing Medical Education presentation with Seattle Cancer Care Alliance (Bremerton, WA)	Gave a presentation to local oncology physicians about our group, Tumor Vaccine Group	November 27, 2006
Booth at Cierra Sisters Conference (Tukwila, WA)	The Cierra Sisters are an African American Breast Cancer Support Group. At this conference we have a booth where we talked to cancer advocates/survivors about immunotherapy and cancer.	February 3, 2007
Downtown Hospital/Cancer Center Visits	Provided information about our group, Tumor Vaccine Group, to local area physician offices interested in receiving information	February 6, 2007
Tumor Vaccine Group Open House (Seattle, WA)	This event was open to the public and was attended by physicians, researchers, cancer advocates/survivors and others interested in the Tumor Vaccine Group. We gave talks, tours and distributed materials about our group.	February 8, 2007
Talk at Cierra Sisters monthly meeting (Seattle, WA)	Gave a talk about immunotherapy and our group in that we are conducting research in breast and ovarian cancers.	February 22, 2007
Eastside Hospital/Cancer Center Visits (Bellevue, WA; Kirkland, WA)	Distributed information about our group to hospitals and cancer centers in the Eastern suburbs of Seattle.	March 23, 2007
Latino Health Fair table and talk (Vancouver, WA)	Gave talk about breast and ovarian cancer. Had a booth where we provided information out immunotherapy and our group.	March 25, 2007
Continuing Medical Education presentation with Seattle Cancer Care Alliance (Everett, WA)	Gave a presentation to local oncology physicians about the work and trials of our group, The Tumor Vaccine Group	April 23, 2007
Continuing Medical Education presentation with Seattle Cancer Care Alliance (Richland, WA)	Gave a presentation to local oncology physicians about the work and trials of our group, The Tumor Vaccine Group	April 25, 2007
University of Washington Health Sciences Open House (Seattle, WA)	Had a booth where we talked to the public about immunotherapy and cancer clinical trials.	April 27, 2007
Continuing Medical Education presentation with Seattle Cancer Care Alliance (Tacoma, WA)	Gave a presentation to local oncology physicians about the work and trials of our group, The Tumor Vaccine Group	May 7, 2007
5th Cross Cultural Conference, "Saving Lives, Eliminating Health Dispartieis" Increasing Minority Participation in Cancer Clinical Trials (Tacoma, WA)	Had a booth where we talked to the public about immunotherapy and cancer clinical trials.	May 17, 2007

During the last reporting period we screened 105 breast cancer subjects with 16 being potentially eligible for this study. We have identified several potential barriers impacting study accrual over the last 6 months and

have addressed some of these barriers by modifying eligibility criteria. These modifications are currently being reviewed by the IRB at the Fred Hutchinson Cancer Research Center, and subsequently will be forwarded to USAMRMC HSRRB and FDA. These protocol modifications include:

- Timing of disease staging: Current criteria states that NED status must be documented by chest/abdominal CT within 45 days of enrollment and bone only disease must be documented as stable or healed by bone scan or MRI within 45 days of enrollment. These criteria, coupled with the requirement that subjects must be enrolled within 6 months of initiating maintenance trastuzumab has hindered our accrual to this study. As per standard of care guidelines, CT imaging is performed on stage IV breast cancer patients no more than every 3 months and further, that bone scans are performed no more than every 6 months.

  In one case, a potential study subject who was being screened in March 2007 had January 2007 CT and bone scans, which were done four months into her maintenance trastuzumab. Because her imaging was only two months old, her insurance would not cover the cost of clinically unnecessary scans and justifiably, she was unwilling to pay out of pocket. If this patient were to have gotten scans sooner than what is dictated by standard of care, she would have unnecessarily been exposed to additional radiation and dyes. In the end this patient was not eligible for study because when her scans were appropriately updated in May 2007, she had been on maintenance trastuzumab for approximately seven months, thereby exceeding the six month limit.

  It should be emphasized that subjects are not encouraged to have additional scans, but are made aware of what documentation is needed to assess their study eligibility.

- Inclusion of ECHO as a modality to assess cardiac function: As per the currently approved study protocol, patients must have a baseline left ventricular ejection fraction (LVEF) measured by MUGA equal to or greater than the lower limit of normal. Given the cardiotoxicity associated with trastuzumab, standard of care dictates that LVEF be evaluated in all patients prior to and periodically during treatment with trastuzumab. Noninvasive modalities commonly used to quantify LVEF include both MUGA and echocardiography. While the MUGA is more commonly used to monitor patients' LVEF over the course of their trastuzumab therapy, echocardiograms are also used and considered acceptable.[1,2] For those followed with echocardiograms, requiring a second measure of LVEF by MUGA often incurs an additional cost to the patient and unnecessarily exposes them to radioactive dye. Additionally, the need for another test to be performed compromises the timing of their eligibility. For instance, a patient who is currently being screened for study and initiated maintenance trastuzumab in April 2007 has imaging from April 2007 and an echocardiogram from January 2007. If echocardiograms were included in the eligibility criteria as a test of cardiac function, she would be considered eligible at present.

- Extend eligibility to Stage IIIB: Given that stage IIIB disease is similar to stage IV in terms of progression-free survival, we have proposed that the eligibility criteria be modified to include patients with stage IIIB HER2 positive breast cancer. To date, this modification has been approved by the FDA.

We currently have 5 subjects in the final stages of collecting source documents (Table 3). The interventions described above have resulted in an increase in subject intake within the last 2 months. We are in the process of establishing relationships with 3 large group practices in the local area with the purpose of specifically increasing enrollment to this trial.

Table 3. Subjects in the Final Phase of Data Collection

Subject	Initial Contact	Source of Referral	State of Residence	Age	Initiation of maintenance trastuzumab
KC	3/30/07	Breastlink	CA	52	3/2007
DG	3/30/07	Breastlink	CA	57	4/2007
EC	4/21/07	Clinician referral (UCSF-Melisko, MD)	CA	46	2/2007
JG	5/10/07	Internet (clinicaltrials.gov)	FL	43	7/2007
TH	5/18/07	Breastlink	CA	47	9/2007

c. Interim statistical analysis after 25 patients have been followed for 1 year. Not applicable for this reporting period. It is understood that once we have enrolled 25 subjects that have been followed for 1 year we should perform an interim analysis of the data.

d. Final analysis of response. Not applicable for this reporting period.

Task 2*: To evaluate the safety of administering a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving trastuzumab monotherapy.*

a. Evaluate immediate toxicity associated with the vaccine. We use the NCI Common Toxicity Criteria for Adverse Events Version 3.0 to grade toxicities. We pay particular attention to local reactions associated with the injection site and systemic reactions to include but not limited to fever, malaise, myalgia, nausea and headache. Table 4 is a comprehensive list of adverse events experienced by all subjects on study.

Table 4. Comprehensive List of Adverse Events Reported

Category	Event	Comments	Grade	Attribution	Expected
Allergy/Immunology	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)		1	2	
Allergy/Immunology	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)		1	2	
Blood/Bone Marrow	Hemoglobin		2	2	
Blood/Bone Marrow	Leukocytes (total WBC)		1	2	
Blood/Bone Marrow	Lymphopenia		1	3	
Blood/Bone Marrow	Lymphopenia		1	3	
Blood/Bone Marrow	Lymphopenia		2	1	
Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)		1	1	YES
Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)		2	2	YES
Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)		2	1	YES
Dermatology/Skin	Dermatology/Skin - Other	eczema	1	3	
Dermatology/Skin	Pruritus/itching		1	3	YES
Dermatology/Skin	Rash/desquamation		1	3	YES
Dermatology/Skin	Rash/desquamation		1	2	YES
Gastrointestinal	Anorexia		1	2	
Gastrointestinal	Diarrhea		1	2	YES

Category	Event	Comments	Grade	Attribution	Expected
Gastrointestinal	Nausea		1	2	YES
Gastrointestinal	Vomiting		1	1	
Infection	Infection -	Port a cath	1	1	
Metabolic/Laboratory	AST, SGOT(serum glutamic oxaloacetic transaminase)		1	3	
Metabolic/Laboratory	Proteinuria		1	3	
Neurology	Cognitive disturbance		1	1	
Neurology	Mood alteration	Anxiety	1	1	
Pain	Pain	Myalgias	1	3	YES
Pain	Pain	Heacache	1	1	YES
Pulmonary/Upper Respiratory	Cough		1	1	
Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other	Rhinitis secondary to Herceptin	1	2	

As part of our Data Safety Monitoring Plan an independent monitor, assigned by the Clinical Trials Support Office (CTSO) at the Fred Hutchinson Cancer Research Center (FHCRC), verifies consent documentation for all newly enrolled subjects in addition to reviewing data collected since the previous monitoring visit for randomly selected subjects. All regulatory documentation is reviewed including all IND documentation. We were monitored for the first time since the first subject was enrolled on February 26-27, 2007. There were no major findings. We are due for monitoring in August 2007.

According to our Data Safety Monitoring Plan (DSMP) we are scheduled to meet with the Medical Monitor and related clinical research staff members on a bi-annual (twice a year) basis. We notified our Medical Monitor of the initiation of the study prior to the first subject starting then followed-up with an official report on January 4, 2007 of the findings of the first vaccine visit of the first subject. Prior to each meeting the Medical Monitor, Dr. Disis and related clinical research staff members will be provided with an agenda, a Bi-Annual Safety and Performance Report which includes total enrollment, adverse event reporting for and recently approved modifications and amendments for the reporting period. All meeting minutes are reviewed, approved and signed by the Medical Monitor before submitting the information to the Fred Hutchinson Cancer Research Center – Cancer Consortium IRB. Our next Data Safety Monitoring meeting is scheduled for June 2007.

b. Determine whether there is any cardiac toxicity associated with the co-administration of the HER2 ICD peptide based vaccine with trastuzumab. When subjects are enrolled we will closely monitor and document any abnormal cardiac events observed by us at clinic visits or reported to us by the subjects or physicians. All subjects have documentation of a MUGA scan within 6 months for eligibility assessment and if that MUGA scan is greater than 60 days old at time of enrollment we perform a MUGA scan at their baseline visit.

A follow-up MUGA scan is performed again at 4 months post-vaccine. As indicated in Task 1.b Table 1 all subjects are currently undergoing the vaccine phase of the study; therefore, we do not have post-vaccine MUGA scans to review as part of the cardiac toxicity associated with the co-administration of the HER2 ICD peptide based vaccine with trastuzumab. We can say; however, that we have not observed nor have any cardiac symptoms been reported to us thus far.

c. Evaluate for any potential toxicities due to the generation of an immune response to HER2. Not applicable to this reporting period.

***Task 3**: To determine the immunogenicity of a HER2 ICD peptide-based vaccine in patients with Stage IV breast cancer receiving concurrent trastuzumab monotherapy*

a. Determine the immunogenicity of the approach by assessing the T cell response to HER2 ICD. We have established a validated cryopreservation method for the storage of lymphocytes. To reduce costs we will analyze all samples from an individual subject at the end of the active vaccination phase. For this reason. There are no data available as yet. All assays proposed have been established and validated as per last years progress report.

b. Determine the incidence of epitope spreading to the HER2 ICD or other peptides in the immunizing mix (intermolecular epitope spreading). Not applicable to this reporting period.

c. Determine the incidence of epitope spreading to other immunogenic proteins associated with breast cancers (extramolecular epitope spreading). Not applicable to this reporting period.

d. Assess the absolute magnitude of the CD4+ and CD8+ HER2 specific immune responses generated after active immunization. Not applicable to this reporting period.

e. Evaluate the generation of HER2 specific antibody immunity and antibody avidity. Not applicable to this reporting period.

f. Determine whether overall survival is associated with the development of HER2 specific T cell response or epitope spreading after active immunization. Not applicable to this reporting period.

KEY RESEARCH ACCOMPLISHMENTS

Not applicable to this reporting period.

REPORTABLE OUTCOMES

None as yet.

CONCLUSIONS

We began study enrollment on December 29, 2006. We have since enrolled six subjects who are at varying phases of vaccination. To date we have observed only low grade adverse events (Grades 1 & 2) most of which were expected and unrelated to the vaccine.

We are aggressively pursuing strategies to increase our accrual to this study by working with our established research and clinical collaborators, and with a variety of breast cancer and health care advocacy groups in the West Coast region. In addition, we have submitted the above-referenced protocol modifications for IRB review, and we anticipate that these modifications will remove significant accrual barriers for this study. Once approved at a local IRB level, we will forward these modifications to the USAMRMC HSRRB and FDA for approval prior to implementation.

In order to successfully accomplish the scope of work for this project, we anticipate the need for a 12 month no-cost extension to allow time to enroll and treat the remaining patients and complete data analysis. We have budgeted our funds accordingly, and will make a formal request for this project extension under separate cover.

REFERENCES

1. Guarneri, V, Lenihan, DJ, Valero, V, et al: Long-term cardiac tolerability of trastuzumab in metastatic breast cancer: The M.D. Anderson Cancer Center experience. J Clin Oncol 24:4107-4115, 2006
2. Ewer, MS, Vooletich, MT, Durand, JB, et al: Reversibility of trastuzumab-related cardiotoxicity: New insights based on clinical course and response to medical treatment. J Clin Oncol 23: 7820-7826, 2005